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Hormonal Effects on Growth of Kinnow Embryo

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Abstract: Kinnow (*Citrus reticulata*) embryos were grown in *in vitro* cultures. Removal of both the testa resulted germination of seeds with in one week while the normal seeds with integuments germinated after three weeks of culture. MS supplemented with BA (1mg l^{-1}), NAA (0.75-1 mg l^{-1}), CH (1 mg l^{-1}) proved to be better for embryo growth. GA_3 and NAA (0.5-1.0mg l^{-1}) was good for root growth. The embryo culture response in MS supplemented with Kn, 2iP and Z were at par with control. The results depicted that Kinnow embryo growth can be improved by BA and CH, while root growth was enhanced by NAA and GA_3 in MS basal medium. However, Kinnow embryo responses were not uniform in repeats because of fruit variability in orchards.

Key words: Kinnow · Embryo · Plant growth regulators · MS medium

INTRODUCTION

Citrus embryogenesis is very important for obtaining plants in different genetic back ground. *Citrus* embryos are produced both *in vivo* in seeds and *in vitro* cultures. *Citrus* embryogenesis has been studied by various researchers [1-6]. The potential of the embryo formation is high in some cultivars but recovery of plants is low. Smaller embryos have been cultured with supplementation of medium by nutritional salts, carbohydrates hormonal additives etc. Isolation of embryos in culture medium to study the factors that control growth and differentiation of embryos is in practice for more than a century [7]. Naturally occurring plant hormones influence physiological processes including growth, differentiation and development in plants. Without hormones, plants would be mostly mass of undifferentiated cells. Phyto hormones control and modify various processes such as seed growth, time of flowering, senescence of leaves and fruits, upward and downward growth of tissues, leaf formation, fruit development and ripening, stem growth, plant longevity and death. Plant growth regulators affect shoot and root growth in *in vitro* cultures. Root and shoot growth was influenced by Kn and NAA concentrations and time length of exposure to *in vitro* grown quince leaves [8]. Plant growth regulators 2,4-D, GA_3 and Kn increased the efficiency of inter specific and inter generic crosses for seed set, embryos per seed and plants germinated per embryo [9]. The putative hormone jasmonic acid (JA) prevent precocious germination of *Brassica napus* microspore embryo and of cultured zygotic embryos at an exogenic concentration of >1 micromolar. This dose response was comparable with results obtained with ABA. Inhibitory effects on seed germination were with JA ad ABA [10]. Salicyl hydroxamic acid stimulated germination of photo sensitive lettuce seeds in darkness by stimulating germination not by enhancing embryo growth potential, but by weakening the micropylar region of the endosperm enclosing the embryo [11]. Abscissic acid controls embryo growth potential and endosperm cap weakening during coffee seed germination [12]. Gibberellins are required for embryo growth and seed development in pea. The results demonstrated that even relatively small changes in the levels of GAs in young seeds can alter seed development [13]. All these reports indicated some role of plant growth regulators in embryo growth. The objective of this study was to find out the effect of growth regulators and nutritional additives like Kinnow juice, coconut milk on Kinnow embryo shoot- root growth.

MATERIALS AND METHODS

Kinnow fruits were collected, washed and seeds were extracted. Seeds were sterilized with 0.1% $HgCl_2$ for five minutes and washed thoroughly with distilled water. Both the seed coats were removed aseptically. The embryos from polyembryonic seeds were isolated and cultured in

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MS basal medium [18] supplemented with different doses (0, 0.25, 0.5, 0.75, 1 and 1.25 mg l^{-1}) of ABA, (0, 0.1, 1, 5 and 10 mg l^{-1}) of GA_3,(0.1, 0.25, 0.5, 0.75, 1 mg l^{-1}) of BA, Kn, Z and 2iP (0, 0.25, 0.5, 0.75, 1 and 1.25 mg l^{-1}) of NAA, (0, 0.25, 0.5, 0.75, 1 and 1.25 mg l^{-1}) of IAA and 0, 1, 5, 10, 15 and 20% of coconut water and Kinnow Juice and 0, 0.5,1, 1.5, 2 and 2.5 g $l^{-1}$of honey. Casein hydrolysate was used as 0.1, 0.25, 0.5, 0.75 and 1g l^{-1} of culture medium. The pH of the medium was 5.8 and the medium was solidified with 1% agar. The cultures were kept in normal day light at 25±2°C. The embryo growth as length of embryo, shoot-root development was recorded after 60 days of culture. For statistical analysis one way ANOVA over six treatments and 20 plant hormones and nutritional additives using MSTAT software [14] was exercised.

RESULTS AND DISCUSSION

The seeds in which the seed coats were removed, germinated within one week and those, in which the coats were sustained, germinated after three weeks. This study was related to the *in vitro* growth of preformed seed embryos. Developing of one primary shoot and root was because of natural apical dominance. Multiple shoots and buds were produced, if there is no apical dominance because of embryo damage or if multiple embryos are naturally fused and grafted at very early embryonic stage. Growth regulators GA_3, BA, Z, Kn, 2iP, NAA, IAA and ABA had some impact on plant growth. MS supplemented with BA, NAA or MS with CH proved better for embryo growth. MS media augmented Kn, 2iP and Z were not better than control response (2.2-6.3 cm embryo length). GA_3 and NAA were good for root growth. There are significant differences among the growth in 20 plant hormones and nutritional additives (Table 1). Higher concentrations of Kinnow juice had inhibitory effects on embryo growth. Honey, when added @ 0.5-2.5 g l^{-1} did not regenerate shoots better than controls nor had inhibited growth of the embryos. Kinnow has variability in all characteristics including fruits, seed shapes, embryony status of seeds etc. Since embryos are derived from cell lines present in branches, therefore, behavior of growth responses of embryos within and between growth hormone treatments was not uniform between repeats and within same flasks, despite careful preparation of culture media. However, the differences among the control (culture in MS basal medium) embryo growth were non significant. The addition of 500 mg l^{-1} malt extract to MS medium increased the germination of early cotyledon stage embryos to 98%. The optimum sucrose concentration to embryo rescue was 150 mille molar. The ability to form plants *in vitro* strongly increased with increasing embryo developmental stage [15]. The factors influencing successful regeneration of nucellar and ovule culture include genotype, age of fruit, presence or

Table 1: Effect of plant hormones and nutritional additives on Kinnow embryo shoot (S.L.), root (R.L.) growth

Sr.N.	Growth regulator	T1	T2	T3	T4	T5
1.	BA S.L	4.0 AB	3.98 A	4.12 B	4.58 B	4.98 A
2.	K. S.L	2.98 DEFG	2.76 DE	2.74 FG	3.22 EFG	3.38 CD
3.	2iP S.L	2.84EFGHI	2.56 DEF	2.6 G	2.96FGHI	3.36 CD
4.	Z S.L	2.92DEFGH	2.92 DE	3.04 EFG	3.18 EFG	3.28 CDE
5.	NAA S.L	2.88DEFGH	2.94 DE	3.1 DEFG	3.24 EFG	3.06 CDE
6.	NAA R.L	3.48 BCD	3.802 A	4.74 A	5.1 A	3.98 B
7.	IAA S.L	2.56 GHIJ	2.5 DEF	2.9 FG	3.2 EFG	3.22 CDE
8.	IAA R.L	2.3 HIJ	2.46 EF	3.22 DEFG	2.58 HIJ	3.14 CDE
9.	ABA S.L	2.54 GHIJ	2.84 DE	2.72 FG	3.24 EFG	2.62 EF
10.	ABA R.L	2.96 DEFG	3.08 BCD	3.18 DEFG	3.42 DEF	2.32 F
11.	GA_3 S.L	2.64FGHIJ	3.48 AB	3.16 DEFG	3.26 EFG	3.12 CDE
12.	GA_3 R.L	2.22 IJ	2.72 DE	3.06 EFG	3.54 DE	3.580 BC
13.	COCONUT WATER S.L	2.72FGHIJ	2.92 CDE	3.88 BC	3.06EFGH	3.22 CDE
14.	COCONUT WATER R.L	3.74 BC	2.92 BCDE	3.36 CDEF	2.46 IJ	2.640 EF
15.	C.H. S.L	3.34 CDE	3.46 ABC	3.6 BCDE	4.06 C	3.0 CDE
16.	C.H. R.L	4.4 A	3.76 A	3.22 DEFG	4.08 C	2.9 DEF
17.	Honey S.L	2.18 J	2.62 DEF	2.88 FG	2.74 GHI	2.72 DEF
18.	Honey R.L	2.66FGHIJ	2.84 DE	3.74 BCD	3.88 CD	3.02 CDE
19.	K.J. S.L	2.14 J	2.1 F	2.64 G	3.52 DEF	2.28 F
20.	K.J. R.L	3.22 CDEF	2.48 EF	2.76 FG	2.1 J	1.34 G

Values followed by same letters in a row do not differ significantly

2iP (mg/l) **GA_3@ 1mg /l** **Embryos growing in pots**

Fig. 1: Kinnow embryos growth in plant hormones and nutritional additives

absence of fertilization and in vitro media composition. Prolonged *in vitro* culture ensures development of vascular connection between the shoots and roots and also the development of an optimum proportion of root to shoot system [16]. The combination of BA@1mg $l^{-1}$for adventitious bud induction in epicotyl segments, followed by rooting in medium with 1mg l^{-1} IBA gave good rooting percentage for Natal, Valencia and Hamlin sweet oranges [17].

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Abbreviations: 2, 4-D: 2, 4-dichlorophenoxy acetic acid; NAA: Naphthalene Acetic Acid; BA: Benzyl Adenine; Kn: Kinetin; IAA: Indole acetic acid; MS: Murashige and Skoog (1962) medium; 2iP: 2-Iso pentenyl aminopurine; Z: Zeatin; ABA; Abcissic acid; GA_3: Gibberellic acid; K.J: Kinnow juice; CH: Casein Hydrolysate